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BY

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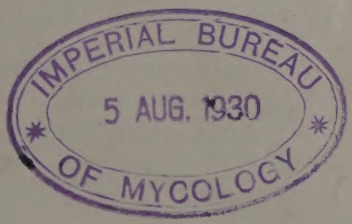

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SOURING OF FIGS BY YEASTS AND THE TRANSMISSION OF THE DISEASE BY INSECTS[1]

By PANOS D. CALDIS,[2] [3]

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INTRODUCTION

Souring was the first disease of the fruit of the fig to be reported from California. For a long time it was the only disease recognized and the term was used indiscriminately to cover all kinds of spoilage. The first reference to this disease was made by Pierce, as reported by Galloway (*11 p. 239–240* [4]) in 1892. He states:

> But another one of the industries of the State which has been greatly extended of late is seriously threatened. This is the growth and curing of figs. It has been observed since the cultivation of this fruit has been attempted that the grower had to contend with a destructive fermentation of the fruit which often caused the loss of nearly the entire crop.

Pierce found that the fruits spoiled, both while on the tree and on the drying trays and that the causal agent was a yeast.

> Numerous experiments with powders and sprayers were used on the trees but with entirely negative results. The cause probably lies in the fact that the fruit is inoculated by insects, the yeast cells being carried by them to the ripening fruit.

No further results were published by Pierce. Howard (*18, p. 93–94*) in 1900 refers to souring in the Smyrna fig (Calimyrna) as follows:

> Souring of the figs was not noticed in the early part of the season, but began later to a limited extent when showers occurred. When the Smyrna figs ripen the ostiolum opens wide and remains open so that a match can easily be inserted and often moderate-sized insects can enter and feed on the sugar. Some of them are caught in the sticky sap and die within the fig. When the figs are ripe and fall ants and beetles of the genera Noxtoxus and Carpophilus enter in this way.

Roeding (*27, p. 50*) in 1903 studied the fig at home and abroad and mentioned souring as occurring in Asia Minor and in California.

> Experience has shown, however, that the Smyrna varieties suffer far less from this trouble than the ordinary sorts. In the orchard of the Fancher Creek Nurseries, where a few of the White Adriatic figs are still growing, from 50 to 75 per cent will sour on the trees, and in adjoining rows of Smyrna figs it is only occasionally that a sour fig can be found.

Eisen (*8*) and Rixford (*26*) also mention souring in an inclusive way. Rixford found tht a closed eye prevents fermentation. Coit and Johnston, as reported by Haring (*17*) in 1921, did not find a specific organism responsible for the decay of figs observed. "Many types of rot were observed, from the soft, watery, fermented type to the typical dry-rot type. Various yeasts, fungi, and bacteria seem to be responsible."

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[1] Received for publication Nov. 5, 1929; issued May, 1930.

[2] Most of the work was done while the writer was the recipient of the James Rosenberg memorial scholarship in agriculture.

[3] The writer wishes to acknowledge his indebtedness to Prof. Ralph E. Smith of the University of California, under whose direction the investigation was carried on, for suggestions and criticisms, and for critical reading of the manuscript.

[4] Reference is made by number (italic) to Literature Cited, p. 1049.

The writer commenced work on this problem in the fall of 1922. It immediately became evident that the decay commonly given the name "souring" was not caused by one agent alone, and that the symptoms were obvious enough to allow the division into at least two distinct diseases. It was furthermore found that the symptoms on caprified varieties, such as the Lob Ingir, Stanford, San Pedro Black, and several unnamed seedlings requiring caprification, were rather of the character of a rot than of a fermentation and were found solely on caprified figs. While fermentation also occurred on caprified

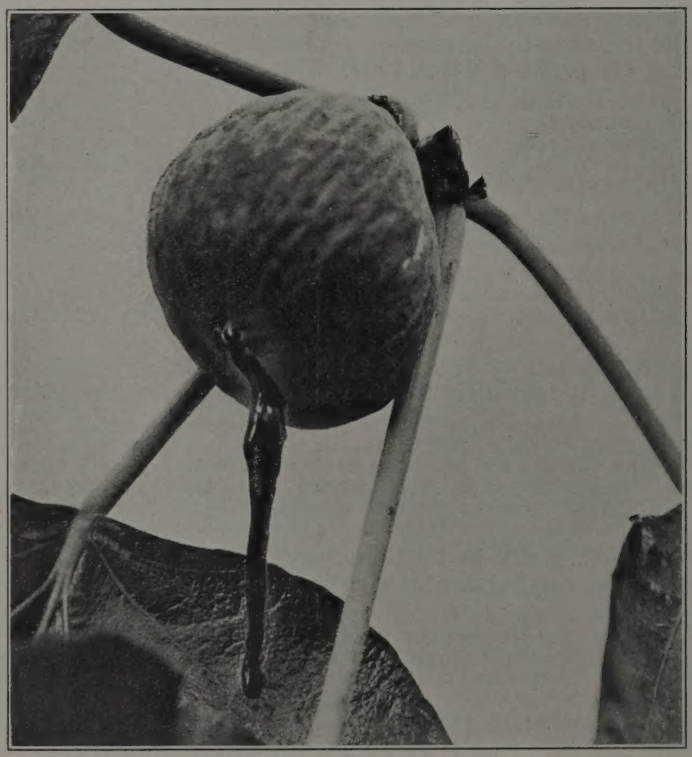

FIGURE 1.—Sour Adriatic fig with jellylike exudate coming out of the eye which indicates that active fermentation is taking place within the fruit

figs, alone or at the same time as the rot, figs of parthenocarpic varieties such as the Adriatic, Mission, Kadota (Dottato), and spring crop San Pedro Black, never exhibited the rot. The findings of the writer on this rot of the fig, termed "endosepsis," have already been discussed (*5*). It is the purpose of this paper to discuss the etiology and transmission of souring.

SYNONYMY AND SYMPTOMS

Souring, as previously mentioned, is the name commonly used to describe all forms of fig spoilage. The term should be restricted to the symptoms described below to cover the spoilage due to fermenta-

tion organisms. Fig fermentation would be a more appropriate name for this disease, but the term "souring" is well established and, when restricted to the disease under discussion, is not confusing.

The symptoms are best observed on fruit of parthenocarpic varieties that have not been caprified, such as the Adriatic. In Lob Ingir and other caprified figs the symptoms are liable to be obscured or confused with the symptoms of endosepsis, or internal rot, that attacks only caprified figs. The symptoms of the disease are manifested only when

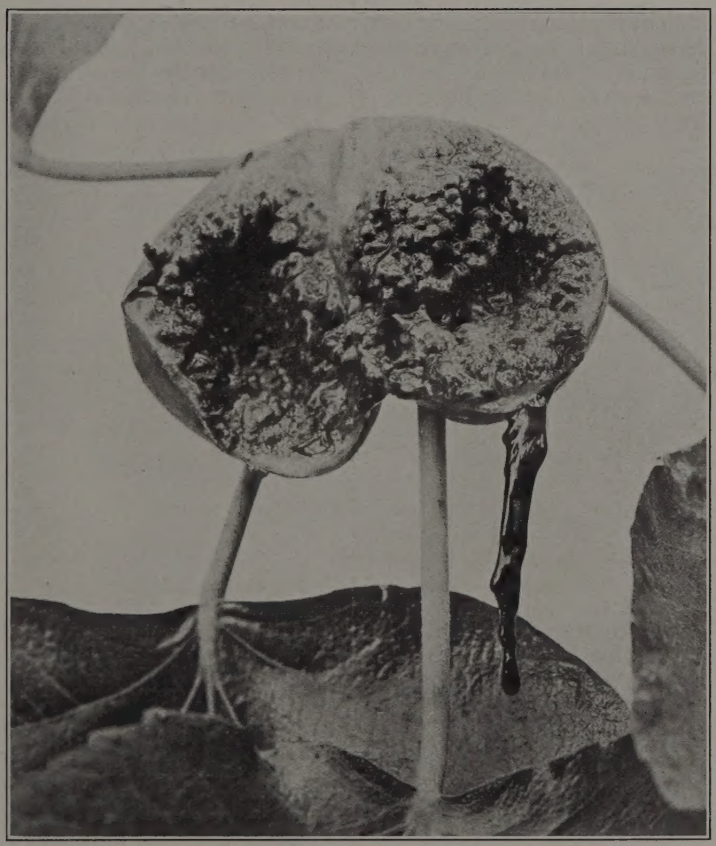

FIGURE 2.—Interior of sour fig shown in Figure 1

the figs begin to ripen and the eye is wide open. In no case has any deterioration been observed in figs of parthenocarpic varieties before the eye opens. Philips et al. (*23*) and Caldis (*5*) have found that the pulp of such figs is sterile previous to the opening of the eye and for considerable time afterwards.

In souring there is at first a change in the color of the pulp, which from pink becomes colorless, and subsequently turns watery. A pink liquid exudes through the eye (fig. 1), dropping on the leaves or jelly-

ing at the eye. Gas bubbles are seen through the pulp and in the skin, and in many cases the pulp becomes water-soaked and loses its firmness. The pulp is disintegrated and smells strongly of alcohol (fig. 2), and is often found to be covered by a white scum. (Fig. 3.) In this condition the figs begin to shrivel and dry up, either dropping to the ground or hanging on the twig, in the latter case giving rise to what is commonly called "black neck" figs. A dead spot or "eye canker" is often formed in the bark at the point of attachment of such figs, as seen in the two twigs at the top of Figure 4. Fermented figs lose their firmness and sag, and usually the pulp becomes detached from the skin at the neck which shrivels, dries up, and turns dark.

Fig souring is primarily an alcoholic fermentation, but subsequent changes may take place while the fig is attached to the twig, on the ground or on the drying board. The commonest change is the action of acetic bacteria on the alcohol with the production of acetic acid,

FIGURE 3.—Yeast scum sometimes found inside sour figs

which is readily discerned by its pungent, strong odor. Ethyl acetate and other esters are probably formed.

DISTRIBUTION AND ECONOMIC IMPORTANCE

Souring has been observed wherever the fig is grown in California. No section is free from it, although orchards have been observed with a very small percentage of figs suffering from this disease. Estimates as to the percentage of injury can not be accurate in the case of Lob Ingirs on account of the coexistence of endosepsis; however, in the case of Adriatics, which are not caprified, souring is very abundant in certain seasons and certain localities and at times increases the cull pile to include the entire crop. There have been cases known to the writer where the crop has not been gathered at all on account of this disease. A grower of Adriatics in the San Joaquin Valley estimated the loss from souring in 1923 as 80 per cent. Such losses are common. Indeed, Adriatic figs seldom escape a high percentage of spoilage from this cause. The reasons for variations from year to year and from locality to locality can best be discussed when the facts regarding transmission have been given. The influence of

the environment will be taken up also at the time, as well as varietal susceptibility.

FIGURE 4.—Dried, sour, "black neck" figs, some of which are still attached to twig; note also dead spot or "eye canker" formed in the bark at point of attachment of such figs

This disease has been observed in foreign fig-growing countries by several travelers, but there is no reference in literature as to importance and extent of damage. Letters of inquiry were written to the departments of agriculture of Italy, France, Greece, Spain, Jugoslavia, Portugal, Turkey, and South Africa in the fall of 1922.

Replies were received only from Italy, Spain, and South Africa. G. B. Traverso, director of the Royal Station of Vegetable Pathology, Rome, Italy, stated that the disease "does not exist in Italy." The director general of agriculture and forestry of Spain replied that "the disease probably exists in Spain * * * but there have been few figs seen with these characteristics, and they never constitute an epidemic" I. Tribolet, of the division of horticulture, department of agriculture, Union of South Africa, stated that he had never seen the disease in that country.

No mention of souring is made by Edgerton (*7*), Gould (*12*), or Matz (*21*) in their studies of fig diseases in the South Atlantic and Gulf States. Siniscalchi (*32*), De Rosa (*28*), Portale (*24*), and Guglielmi (*13*) make no mention of souring among the fig diseases enumerated as occurring in Italy. Ferrari (*10*), writing about the fig industry in Cosenza, Italy, describes a disease of the fruit in connection with a bacteriosis of the tree. He states that the figs attacked by the disease when nearly ripe show a drop of liquid at the eye, first yellow, then reddish, which increases in volume and, if the infection is heavy, is exuded. This may be souring, but there are no definite data given. Vallese (*36*) also mentions a disease of the fig in Italy that may be similar to souring. Trabut (*35*) and Guillochon (*14*) from North Africa and Esterlich (*9*) from Spain do not mention souring at all in their papers.

Condit, in an unpublished report on the fig industry in Europe, Asia Minor, and north Africa, states the following about fig souring:

> In my report on my trip to Europe I find the following regarding fig souring: The fact undoubtedly is that fig souring occurs more commonly than the Europeans like to admit. The fancy Smyrna layer or Locoum figs, the Greek string figs, or the Spanish fleur figs, which are seen in city markets, represent only the best part of the crop. For example, fig merchants of Smyrna prefer the crop from the hillside orchards of the upper Meander Valley because the figs in the Sokia district and especially in the Ayassouluk or coastal district are soft, sour, dark colored, and inferior. It is a well-known fact that the largest proportion of the fig crop of Mallorca Province, Spain, is used for hog feed or distilled on account of its poor quality. The packers of Coin, Malaga Province, Spain, state that the growers deliver only 50 per cent of the crop to the packing house, feeding the other inferior half to cattle and hogs. Immense quantities of sour and rotten or inferior figs are shipped from Southern Italy to Trieste or Vienna for coffee factories. I myself found Smyrna figs souring at Ayassouluk on August 20, but not in serious quantities. At Kalamata figs of a garden variety were souring badly in the yard of Mr. Pantaxopoulos near the seashore on September 4. Rain-damaged figs were very abundant throughout southern Europe this season, but these could hardly be classed as sour figs. The lateness of the season in Spain and Portugal prevented actual observations along this line.

ETIOLOGY

FORMS OF THE YEASTS ISOLATED

Pierce (*11, p. 240*) isolated

> a yeast, which when pure cultures were made, was applied to fruit on both trees and the drying board. The result was the production of an exactly similar fermation to that occurring naturally.

No identification of the organism is reported. Coit and Johnston (*17*) could not assign a specific organism to the decay. They found various yeasts, fungi, and bacteria apparently responsible for the disease.

When fruit not affected with endosepsis was examined and showed the symptoms described previously, especially the early ones, the

author has invariably isolated pure cultures of yeasts. The disease was studied mostly on figs of the Adriatic variety. This variety is very susceptible to souring, it is widely planted in California and, when it is not caprified, endosepsis does not affect it. There is more than one species of yeast responsible for this disease. It is conceivable that every true yeast introduced into the cavity of the fig, when the latter is ripe, the cavity full of sugary solution and the concentration not too high, would be capable or exciting fermentation. In many platings of sour Adriatics, however, it was found that principally two forms of yeasts were invariably present. A third one was also present but was found incapable of producing the disease. All three forms belong to the class of asporogenous or wild yeasts. The form or forms most often found is a top yeast that produces a scum in liquid media and in the fig cavity and belongs most probably to the Mycoderma class. (Fig. 5.) The second form often present is a bottom yeast, growing poorly on artificial media, especially liquid ones, and belonging to the Apiculate class. The third form is a round yeast of the Torula type. These forms are hereafter designated as A (Mycoderma), C (Apiculate), and E (Torula). Single-cell isolations of these yeasts made by the micropipette method were used in inoculations and in studying their fermentation and other physiological activities.

CULTURAL CHARACTERISTICS OF THE YEASTS ISOLATED

Two Per Cent Dextrose Broth

Yeast A: A thin veillike pellicle is produced on the surface of the liquid in 24 hours and climbs up the sides of the tube. The liquid is cloudy with an abundant sediment. Copious gas is evolved on shaking the tube.

Yeast C: A scarcely noticeable precipitate is formed at the bottom of the tube. This yeast grows very poorly, if at all, in the liquid media used, except in grape juice. A brownish pigment is produced at room temperature (21° C.) and at 28° C. No pigment at lower temperature.

Yeast E: Cloudiness and abundant precipitates are produced, but no pellicle. A small amount of gas produced by some strains.

Two Per Cent Dextrose Nutrient Agar

Yeast A: An abundant, spreading, dull white, somewhat powdery growth is produced, the margin is lobate, with ciliate edge more evident on plates. (Fig. 5.) The center of the stroke is raised, whereas the margins are filmlike.

Yeast C: Very scanty, wet, transparent, beaded growth is produced, with a brownish pigment at room temperature.

Yeast E: Abundant, spreading, white, shiny butyrous growth, with smooth margin.

Malt Agar [5]

Yeast A: Abundant growth, dull white, less than on dextrose nutrient agar.

Yeast C: Scanty whitish growth.

Yeast E: Abundant shiny white growth.

[5] One hundred grams of germinated barley was ground up and 1,000 c.c. of water added. The mixture was heated at 55° to 58° C. for one hour and held over for 24 hours. It was then boiled and filtered, agar added, was tubed and sterilized.

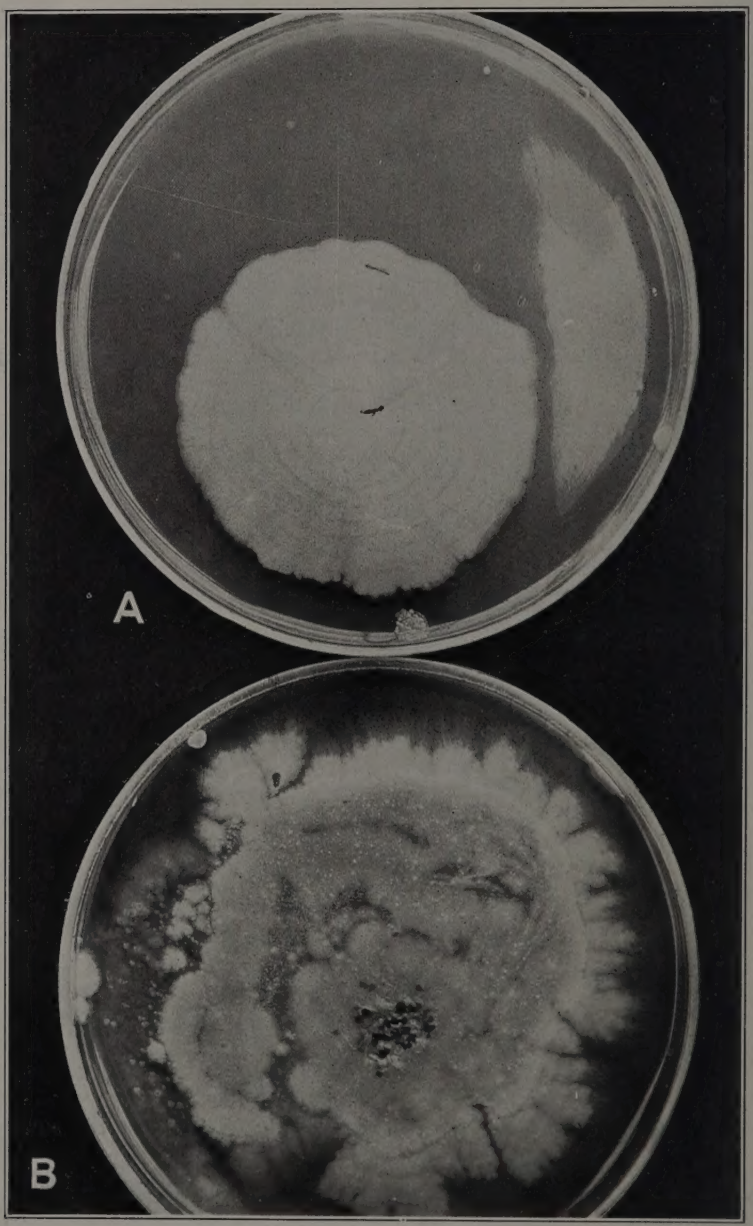

FIGURE 5.—Cultures of yeast A obtained (A) from dried-fruit beetle and (B) from the internal tissue of a sour fig

Four Per Cent Dextrose Laurent Liquid

Yeasts A and E: As in dextrose bouillon.
Yeast C: No growth.

Giant Colonies on Two Per Cent Dextrose Nutrient Agar Plate

Yeast A: Growth rapid, irregular, dull white, with rough surface, effuse elevation, margin lobate, cilliate. (Fig. 5.)
Yeast C: Slow growth, round to irregular, with flat, smooth surface, undulate to lobate edge.
Yeast E: Medium growth, round colony, three-fourths of an inch in diameter, white, shiny, elevation effuse, surface ringed, edge entire.

Laurent Agar Plate

Yeast A: Large irregular colonies, dull white.
Yeast C: Small transparent white colonies.
Yeast E: Small, shiny white round colonies.

Fig Infusion [7]

Yeast A: Thin pellicle, liquid clear, brownish sediment, thick ring.
Yeast C: Slight ring, no pellicle, brown sediment.
Yeast E: As in A.

Fermentation of Sugars by the Yeasts Isolated

Glucose, fructose, sucrose, lactose, and maltose have been tested, as well as apple cider, grape juice, and fig infusion. The sugars were added either to beef bouillon, to Laurent solution, or to the ammonium phosphate medium used in sugar fermentation with bacteria (*33*). Smith and Dunham fermentation tubes were used.

Nine isolations of yeast A, three of yeast E, and four of yeast C were inoculated into Smith fermentation tubes containing 2 per cent dextrose, fructose, sucrose, maltose, lactose bouillon, cider, Zinfandel grape juice, and fig infusion.

It is evident from Table 1 that the disaccharides are not fermentable by the three yeasts isolated from figs. Gas was produced from dextrose and fructose to a great extent by yeast A. Yeast C produced a small amount of gas from fructose 30 days after inoculation. Yeast E also produced a small amount. Gas was produced abundantly by the three yeasts from grape juice, and by yeasts A and C from fig infusion, but not by yeast E. Pellicle, a cloudy bulb, but a clear arm was produced by yeast A on all the above media. Maximum gas was produced by yeast A on all the media. Maximum gas was produced in 10 to 12 days. The nine isolations of yeast A preduced variable percentages of gas from dextrose in the closed arm of the fermentation tube. The percentages varied from 4.7 to 23.5 per cent, an average of 14.4 per cent produced in from 5 to 8 days. The greater percentage of gas was produced in 8 days, the next (23.5 per cent) in 5 days, while the lowest amount of 4.7 per cent was produced in 10 days.

[6] Composed of 4.71 gms. ammonium sulphate, 0.75 gm. potassium phosphate, 0.1 gm. magnesium sulphate, 1,000 c. c. water and 2 per cent of the sugar to be studied.
[7] One hundred grams of dried figs boiled in 1,000 c.c. of water for one-half hour, filtered and sterilized Agar added, if wanted.

TABLE 1.—*Results of fermentation tests of various sugars and fruit juices with yeasts obtained from sour figs*

Medium	pH	Yeast A	Yeast C	Yeast E
2 per cent dextrose broth	6.4	Abundant gas	Slight growth, no gas	Small amount gas, slight growth.
2 per cent fructose broth	6.4	do	Slight amount in 30 days	No gas.
2 per cent sucrose broth	6.6	No growth	No growth	Small amount gas.
2 per cent maltose broth	6.5	do	do	No growth.
2 per cent lactose broth	6.5	do	do	Do.
Apple cider	3.4	Small amount gas	do	Do.
Zinfandel grape juice	3.9	Abundant gas	Abundant gas	Abundant gas.
Fig infusion	4.8	do	do	No gas.

Zinfandel grape juice was found to be a favorable medium for all these yeasts. To test their fermentation abilities, 200 c. c. of grape juice was placed in 500 c. c. Erlenmeyer flasks and sterilized by steaming for three consecutive days. They were then inoculated in duplicate with three single cell isolations of yeast A made by the micropipette method, one of yeast C, and one of yeast E. The flasks were weighed daily, and when the loss of weight was comparable to that of the check, a portion of 100 c. c. was distilled with the addition of 50 c. c. of water. The three strains of yeast A produced an average of 7.10 per cent of alcohol by volume (4.7 per cent by weight). Yeast C produced 8.22 per cent and yeast E 6.55 per cent (6.6 and 5.5 per cent, respectively, by weight).

For another test of the fermentation and acid production by yeasts A and E, ammonium phosphate medium and beef-extract broth, both solid and liquid, with the addition of brom cresol purple indicator, were used with each of the following sugars: Dextrose, sucrose, and lactose. The solid media were slanted, the liquids were put in Dunham fermentation tubes. Yeast A produced both acid and gas from the dextrose synthetic, and gas but no acid from the dextrose bouillon. Neither acid nor gas was produced from either the liquid or solid, the synthetic, or the bouillons with the addition of sucrose and lactose. Yeast E produced acid on the liquid synthetic dextrose, but no gas. A small amount of gas, but no acid, was produced after 13 days from dextrose bouillon. Acid and gas were produced from sucrose, both in the synthetic and the broth media. Neither acid nor gas was produced from lactose in either type of medium. The production of gas by yeast E was found to be irregular. Of the three isolations made, which otherwise appear identical, one produced small amounts of gas from glucose and grape juice, the other two did not.

As mentioned previously, the yeasts isolated from figs act indifferently toward disaccharides. It is of interest that figs do not contain such sugars. Leclerc du Sablon (*30*) found very little nonreducing sugars in the varieties Dorée (Figue d'or), Datte Quodidienne (Figue datte), and Barnissotte Black (Bourjasotte noir). An analysis for sugars of healthy and diseased Adriatic figs was made by the writer in 1923. Healthy figs picked fresh contained 69.61 per cent of reducing sugars soluble in alcohol, while sour figs, picked as they dropped from the tree, contained 59.45 per cent of reducing sugars soluble in alcohol, both percentages calculated on a moisture-free basis. No nonreducing sugars were found in either the healthy or the diseased figs.

To demonstrate that this disease is actually a fermentation, the following experiment was tried: Sour Adriatic figs were selected from

the drying board and examined for the typical symptoms of the disease. Eight hundred grams of the figs were macerated with 500 c. c. of water and steam-distilled for three hours. The first portion of the distillate was neutralized with potassium hydroxide and redistilled. Fifty seven cubic centimeters of distillate was obtained, with a specific gravity of 0.9812, or 13 per cent of alcohol by volume. The second fraction was also neutralized and redistilled, 250 c. c. was obtained, with a specific gravity of 0.9950, or 2.20 per cent of alcohol by volume. Sour Adriatic figs picked from the ground as they were dropping from the trees, were treated as above and 3.55 per cent of alcohol by volume was obtained. A peculiar, ethereal odor passed into the distillates.

Rossi (*29*), in his extensive review of the literature regarding apiculate yeasts, mentions that *Pseudosaccharomyces apiculatus* isolated from grapes produces 3.15 per cent of alcohol by weight from dextrose. He states that acetic and formic acid as well as esters and other volatile substances were found by Amthor, Müller-Thurgau, Kayser, Seifert, and Mach e Portele to be produced by this yeast. It has been mentioned already that sour figs at times have an ester smell.

MORPHOLOGY OF THE YEASTS ISOLATED

Yeast A: A top yeast with long and narrow cells varying considerably in length. Budding apical, the daughter cells remaining attached to form long chains with as many as three daughter cells attached to the same apex. A few cells are slightly curved. The cells are vacuolated, especially on solid media. No spores are produced on plaster blocks, Gorodkowa's medium (*20*) or carrot plugs. The cells are 6 to 33 by 2.2 to 6 μ (average 8.9 by 3.9) on dextrose nutrient agar and 7.5 to 31.5 by 3 to 6 μ (average 12.4 by 4.7) on dextrose bouillon. (Fig. 6.)

Yeast C: Typically apiculate bottom yeast measuring 4.1 by 2.0 μ, single or in pairs, sometimes elliptical, never in threads. No spores found. (Fig. 6.)

Yeast E: A bottom yeast with almost perfectly round cells, with a large vacuole, budding freely, the daughter cells remaining attached for some time forming chains of three or four cells. No spores are produced on plaster blocks, Gorodkowa medium or carrot. Budding cells measure 4.19 by 3.4 μ and 3.9 by 3.9 μ when not budding. Some strains are smaller, measuring 3.1 by 3.1 μ. (Fig. 6.)

PATHOGENICITY OF THE YEASTS ISOLATED

Inoculations with single-cell cultures of these yeasts were made on fresh figs that were still attached to the tree. Figs were selected with the eye fairly well closed and inoculated by inserting a needle through the eye.

The whole twig was then bagged with a 3-pound manila paper bag and tied firmly in order to exclude insects from the fig. (Fig. 7.) Inoculations were made in triplicate. The eyes of the figs inoculated were previously swabbed with alcoholic mercuric chloride. The figs when ripe were brought to the laboratory and plated.

Yeast A: Typically sour figs were obtained by inoculating with this yeast. The pulp of the figs was discolored and covered by a white scum. One side of the fig was softened and water-soaked. The figs were often bloated, gassy, and sagging, with the pulp drawn away from the stem end. Slight dripping was observed. When green figs were inoculated, the results were sometimes negative.

Yeast C: Fermented figs were obtained with this yeast essentially as with yeast A, but without the scum on the surface of the pulp cavity.

Yeast E: Inoculations with this yeast did not yield typically sour figs. The pulp of the fig was discolored and gelatinized with an odor approaching that of sour figs.

TRANSMISSION

Berlese (*2*, *3*, *4*) has found that yeasts are very scarce in the air during late spring and early summer, but abundant on trunks and

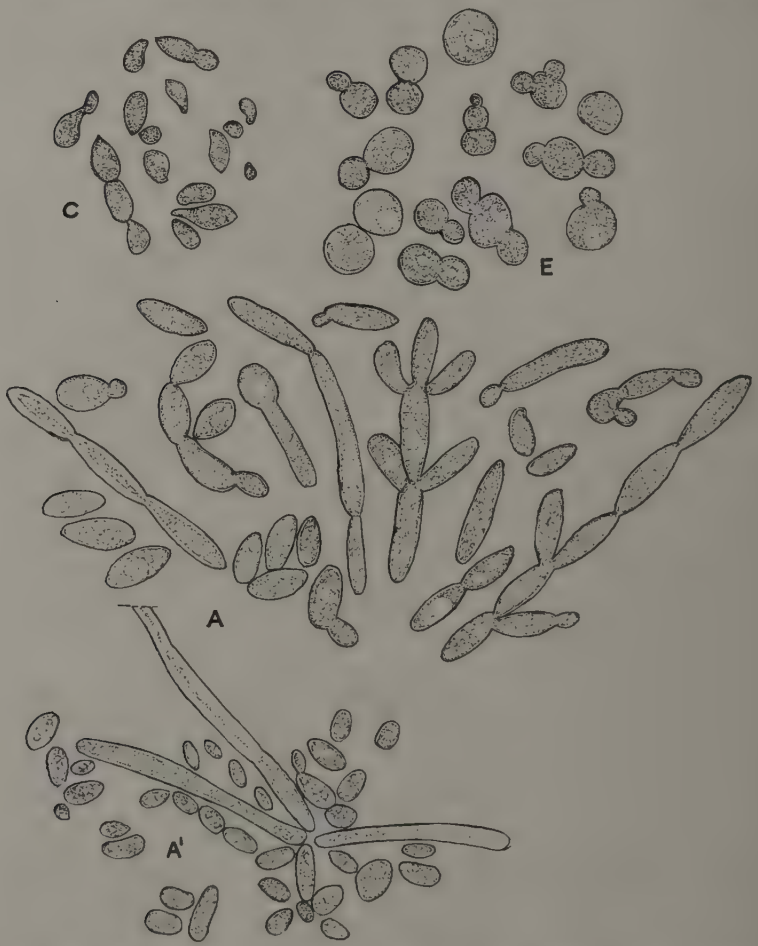

FIGURE 6.—Yeasts isolated from sour figs. Camera lucida drawings × 1,400 from 7-day old cultures. Yeasts A and E grown on Laurent liquid, C on dextrose nutrient agar. A', pellicle of yeast A on dextrose bouillon

other parts of trees. He found *Saccharomyces apiculatus*, *S. ellipsoideus*, and *S. pastorianus* in the soil from April to June, but rarely afterwards. Insects, however, such as ants and flies (*Sarcophaga carnaria* L., *Drosophila cellaris* L., *Calliphora erythrocephala*, Dasyphora, Crysotoxum, Eristalis, Aricia, Lucilia, and *Anthomyia* sp.),

were found carrying yeasts both externally and internally. Yeasts were found to multiply in their intestines and to hibernate in them.

Phillips et al. (*23*) in their studies on fig smut found that exclusion of insects from two large fig trees by building a tent of muslin cloth over the trees reduced the amount of souring to nothing, while there was a large number of sour figs on the neighboring trees. They also found that the insects most common in figs still on the tree were the dried-fruit beetle, *Carpophilus hemipterus* L., and the vinegar fly, *Drosophila ampelophila* Löw. It may be deduced, therefore, that these two insects may be concerned in the transmission of the yeasts causing souring into the cavity of the fig. In order to test this theory, a number of sacks were made of brass strainer cloth 1 by 1.5 feet,

FIGURE 7.—Figs covered with paper bags to exclude insects in experiments on the transmission of souring by inoculation with yeasts

soldered on top and side. These sacks were slipped over fig twigs bearing from 3 to 9 figs, fully grown but green, whose eye scales were tightly closed. The sack was sewed at the bottom, and a large plug of cotton placed in the opening through which the twig was introduced. One hundred manila paper sacks were also placed on similar figs. (Fig. 7.) Adriatic figs were used in this experiment in 1924; six strainer-cloth sacks were placed on August 1 and six more on August 26. When the figs commenced ripening in the sacks (September 8) 12 dried-fruit beetles, collected from figs in sterile vials by holding the mouth of the vial over the eye of the fig and tapping the sides so that the beetles emerged from the fig and entered the vial, were introduced into each of four of the sacks. The beetles were watched awhile, and they were seen crawling on the figs. Many sour figs were on the

trees on which the sacks were placed. When every fig in the sacks ripened and dried up, the twigs bearing the sacks were cut and brought to the laboratory for examination. The results are given in Table 2.

TABLE 2.—*Effect upon souring of exclusion and introduction of Carpophilus hemipterus L. into strainer-cloth sacks inclosing ripening Adriatic figs*

No beetles introduced			12 beetles introduced in each sack				
Sack No.	Total figs	Sour	Sack No.	Total figs	Sour	Wormy but not sour	Sound
1	5	None.	4	7	3		3
2	4	None.	6	9	3	6	
3	9	None.	7	5	2		3
5	8	None.	12	5		5	
8	4	None.					
9	6	None.	Total	26	8	11	6
10	3	None.					
11	6	None.					
Total	45	0					

The beetles fed and bred in many of the figs, as many more beetles were found in the sacks than the number introduced. Larvae were also found in them. No insects of any kind were found in the eight sacks where they were not purposely introduced. The results indicate that the beetles enter the figs readily but do not cause fermentation unless they are themselves infected. In many cases the wormy figs had been sour, but the alcohol had evaporated in drying, leaving a seedy, dry fig, wormy, devoid of sugar but not sour in the ordinary sense. This has been observed also in the orchard. As mentioned previously, the spoilage may stop at any stage in its development, depending on drying conditions, moisture, and presence of the proper organism.

The beetles from the different sacks were collected and cultured in tubes of dextrose bouillon. The sour figs were plated. The results are as shown in Table 3.

TABLE 3.—*Results of making cultures from Carpophilus hemipterus L. found in sacks containing sour figs*

[Cf. Table 2]

Sack No.	Number of beetles	Number infected with yeasts	Type of yeast
4	3	1	C
6	8	3	A
7	7	7	C
12	8	0	

From these experiments it may be concluded that *Carpophilus hemipterus* L. is a transmitting agent of yeasts and bacteria into the figs, where they set up fermentation if the sugar concentration is favorable. To determine the actual flora carried by the dried-fruit beetles, and especially whether the transmission is internal or external, beetles were collected at different times from fig orchards by holding

the mouth of a sterile vial against the eye of an infested fig and tapping the sides. The beetles entered the vial and were thus taken to the laboratory alive and uncontaminated. Drosophila were caught in the same way. The insects were studied as follows: (1) Caught with sterile tweezers and dropped into sterile tubes of dextrose bouillon. One beetle was thrown into each tube. The beetles were either crushed or dropped according as they were dead or alive; (2) placed in Gooch crucibles and disinfected by immersing in 15 per cent solution of potassium hydroxide for 2 minutes, then in 1:500 solution of mercuric chloride for 3 minutes, and washed finally with sterile (autoclaved) water until the washings were free of chlorides as determined by testing with silver nitrate solution; momentary dipping in 95 per cent alcohol was at times used, instead of potassium hydroxide, and 1:1,000 alcoholic (50 per cent) solution of mercuric chloride, instead of the 1:500 solution. No difference was observed in the two methods of disinfection, both being equally effective. After disinfection, the beetles were dropped into tubes of sterile dextrose bouillon, one beetle per tube, left in this tube for varying lengths of time, 1 to 20 minutes, and then removed by means of a sterile platinum loop and dropped into another tube where they were left, The purpose of the shaking into the first tube for varying lengths of time was to obtain a check on the effectiveness of the external sterilization. Unsterilized beetles were shaken into one tube first and transferred into a second tube afterwards in order to determine whether there was any difference between their external and internal flora. Different lots of beetles were tested separately, and beetles were also collected from decaying watermelons, which serve as overwintering grounds for these insects, as was found by Phillips et al. (*23*) and the writer. The results of these tests are summarized in Tables 4 and 5.

TABLE 4.—*Results of various culture tests on Carpophilus hemipterus L., sterilized and unsterilized, as a carrier of yeasts*

UNSTERILIZED

[Figures refer to numbers of insects cultured]

Treatment of beetles	Carpophilus hemipterus L. secured from—																				
	Figs												Watermelons			Figs					
	Aug. 30, 1924			Sept. 12, 1924			Oct. 6, 1924			Oct. 30, 1924			Nov. 11, 1924			Oct. 27, 1925			Nov. 5, 1925		
	Type of yeast																				
	A	C	Negative	A	C	Negative	A	C	Negative	A	C	Negative	A	C	Negative	A	C	Negative	A	C	Negative
Dropped in bouillon and left	2		1	9	3	1	4	8	13	2	4	3	4	9	4	5	6	11	5		23
Left for 1 to 19 minutes, then removed	14			4	2						1	2	1		3		2	2			5
Insects from last		[a] 2	2	1						1		1	1	1	2		3				

STERILIZED

Dropped in bouillon and left (uncrushed)	5		5		5	3					3	6		1	3	1	1	5	1		4
Dropped in bouillon and left (crushed)	4	6	6	4	2	3						8		2	1			13	1		11
Left for 1 to 20 minutes then removed (shaken)			5			9						16			10			24			11
Insects from last	4			2	2	5					3	15	2	3	6		2	22			11

[a] The insects were sterilized after removing from tube 1.

TABLE 5.—*Summary of Table 4*

UNSTERILIZED

Item	Beetles carrying yeast A		Beetles carrying yeast C		Beetles negative		Total beetles used
	Number	*Per cent*	*Number*	*Per cent*	*Number*	*Per cent*	
1. Dropped in bouillon and left	31	26	30	26	56	48	117
2. Left for 1 to 19 minutes, then removed	19	53	5	14	12	33	36
3. Insects from 2	3	21	6	43	5	36	14

STERILIZED

1. Dropped in bouillon and left (uncrushed)	70	16	10	23	26	61	43
2. Dropped in bouillon and left (crushed)	9	15	10	16	42	69	61
3. Left for 1 to 20 minutes, then removed (shaken)					75	100	75
4. Insects from 3	8	10	10	13	59	77	77

The fig tissues yielded the usual yeasts, yeast C and yeast A. In sack 12, where none of the figs soured, the beetles were found to be free of yeasts. Different bacteria were also obtained from the beetles. The figs inclosed in manila paper sacks at the same time as the strainer cloth sacks were also found to be in perfect condition.

This experiment was repeated in 1925 at Davis, Calif., figs of the following varieties being used: Adriatic, Verdal, and Mission, using 20 sacks, which inclosed a total of 147 figs. Of these, 29 figs (3 sacks) were exposed to dried-fruit beetles. Nine of them were found wormy and on plating yielded the usual yeast. The figs were dry and seedy, smelling faintly of alcohol. The remaining 20 figs were not sour. The sacked figs which were not exposed to beetles (17 sacks, 118 figs) were all found to be in perfect condition.

Rand and Pierce (*25*) in 1920 have reviewed the literature on the subject of insect transmission of plant and animal diseases. Insects transmit pathogenes in three ways: (1) Mechanically, by picking up the spores on the exterior of their bodies and accidentally sowing them on the surface or inoculating them into punctures; (2) by making avenues of infection through wounds; and (3) by transmitting them internally, either mechanically or biologically.

The results in Table 4 show that both forms of yeasts isolated from fermenting figs are carried by the dried-fruit beetle. When the beetles were dropped in tubes of broth and left for different intervals of time, 1 to 10 minutes, and then removed, fermentation was set up in many cases (67 per cent), indicating that the types of yeasts mentioned previously are carried mechanically externally. The elytra, the legs, the thorax, and the abdomen were removed and tested separately, and were all found carrying the yeasts. Fermentation was also caused by beetles dropped into bouillon tubes and left uncrushed or crushed. When the beetles were previously sterilized by one of the methods described and then dropped into tubes of bouillon and left uncrushed or crushed, fermentation was also set up. When such beetles were shaken for a limited length of time, 1 to 20 minutes, in a tube of broth and then transferred to another tube where they were left crushed or even uncrushed, there was never fermentation or any kind of growth in the first tube, but in many cases (23 per cent) fermentation was set up in the second tube, indicating that transmission is also internal mechanical. No attempt was

made to discover whether the transmission is internal biological, i. e., whether the yeasts actually multiply in the intestines of the beetles. Berlese (*4*) has shown this to be the case with flies. Both types of yeasts were found to be carried at the same time, and in one case one yeast was found carried externally and the other internally.

As the season advanced a larger number of beetles were found not carrying yeasts. Different bacteria and especially a short rod in chains were usually associated with the yeasts or obtained as the sole flora. *Aspergillus* sp., *Rhizopus* sp., and *Penicillium* sp. were also obtained from beetles, the first by far the greatest number of times.

The opinion has been frequently expressed that vinegar flies, *Drosophila* sp., are attracted to fermenting figs but do not themselves enter healthy ones and so spread the disease. The writer has observed them to enter or issue from figs which when examined afterwards were apparently healthy. Vinegar flies were caught in the same way as dried-fruit beetles and were found carrying the yeasts both externally and internally.

While the flies are attracted by the odors of fermentation and are known to feed on yeasts and decaying fruit, as reported by Baumberger (*1*), Schulze (*31*), Sturtevant (*34*), and Northrop (*22*), Baumberger has found that adult Drosophila flies oviposit on sterile fruit, the presence of yeasts being unnecessary, and Guyénot (*15*, *16*) has raised *Drosophila ampelophila* aseptically for two years (40 generations), indicating that Drosophila flies may enter sound fruit for the purpose of breeding and feeding and in so doing transmit the fig-souring organisms.

Phillips et al. (*23*) in their studies of the transmitting agents of *Aspergillus niger* causing what is called black smut in figs consider *Carpophilus hemipterus* first in importance and *Drosophila ampelophila* as second. The habitats of the dried-fruit beetle throughout the year are discussed. The writer has made observations which confirm the results obtained by Phillips. The appearance of the first sour figs in many orchards coincided with the appearance of the first beetles, and in frequent trips through the figs district of the State isolated orchards have been studied for the presence of both the beetles and souring. It seemed that wherever decaying fruit, melons, oranges, figs, apples, apricots, peaches, plums, etc., were left on the ground, offering a breeding place for the beetles and the vinegar fly, souring was invariably abundant. Young Adriatic fig orchards in sections where grain farming predominated were found free of souring and beetles It was interesting to note the souring of the first crop figs of Adriatic, Brunswick, Mission, Kadota, and White San Pedro. These come early in the season (June) when the overwintering generation of the beetle has not multiplied extensively; souring, therefore, is found only in such figs in orchards with decaying oranges, melons, or other fruits having imperfect sanitation. In connection with orchard sanitation and the presence of souring, an inquiry was made by I. J. Condit about the presence of the dried-fruit beetle in Italy where souring is reported as nonexistent. Doctor Briganti, of Portici, Italy, wrote that "*Carpophilus hemipterus* is rarely found in Italy * * * cases of fig smut and the manifestation of the fruit beetle are so sporadic and of so slight an importance as not to interest deeply our entomologists." In a populous country like Italy, fruits decaying under the trees or on the drying grounds are very scarce.

The idea has been often expressed by Eisen (*8*), Coit (*6*), Coit and Johnston (*17*), and is prevalent among growers that cold nights, overirrigation, cool damp weather, or other environmental conditions cause souring. In view of the evidence presented above, moisture variations can have no other than a modifying effect. Given the infection, carried by the dried-fruit beetle or the vinegar fly, slow drying of the fig with the accompanying slow increase in sugar concentration may favor greatly the development of the parasite. The opposite would be the case with quick drying. The yeasts would probably never develop or their activities would be quickly checked. To find out the effect of high sugar concentration on the growth of yeasts, nutrient agar was prepared to which 40 and 60 per cent of dextrose were added, instead of the customary 2 per cent. The yeasts grew readily on 40 per cent but made very poor growth on the 60 per cent dextrose agar.

SUMMARY

A destructive fermentation of figs, both caprified and parthenocarpic, is described, with a discussion of the economic importance of the disease and its geographical distribution.

Three different types of yeasts were isolated from fermenting figs; their cultural characteristics, fermentation ability, morphology, and pathogenicity are discussed.

The transmission of these yeasts into the cavities of figs by the dried-fruit beetle (*Carpophilus hemipterus*) is shown. The transmission was found to be both internal and external mechanical.

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